COVALENT COUPLING OF AMINOACYL-tRNA TO MODIFIED CELLULOSE AS A METHOD OF PURIFICATION OF SPECIFIC tRNAs*

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1. Introduction

The commonly known similarity of different tRNAs [1,2] usually makes the isolation of a single species tedious and time-consuming [2–5]. The most often employed methods of preparing the specific tRNAs involve several purification steps and diverse chromatographic techniques [6,7] and only a few single tRNAs have been isolated readily thus far [8–10].

The present paper describes a simple method based on selective aminoacylation, binding of the aminoacyl-tRNA to modified cellulose and subsequent liberation of the specific tRNA by hydrolysis of the ester bond between the matrix-fixed amino acid and terminal adenosine of tRNA.

The specific aminoacylation has been exploited in several methods [6, 11–13] of tRNA purification. The only tRNA isolated by covalent coupling of aminoacyl-tRNA to a solid carrier, however, is $tRNA^{Cys}$ purified by binding cysteinyl-$tRNA^{Cys}$ to an organomercurial-polysaccharide [13].

Covalent binding of nucleic acids to solid matrices can be done in many ways. The direct binding of RNA to CNBr-activated Sepharose [14], coupling of RNA oxidized at terminal nucleoside to hydrazide-type supports [11,15,16], esterifying of cellulose with carbodiimide-activated phosphate groups of nucleic acids [17,18] as well as UV-activated binding of DNA [19] to cellulose were employed. The method of AA-tRNA binding used in this paper is essentially that described by Bartkowiak and Pawełkiewicz [20] for the preparation of a carrier suitable for purification of isoleucyl-tRNA synthetase.

2. Materials and methods

2.1. *Crude tRNA and aminoacyl-tRNA synthetase*

Crude tRNA was isolated from ground barley or lupin seeds by phenol extraction [21], purified on DEAE-cellulose, freed of high molecular weight RNA and deaminoacylated as described by Vanderhoef et al. [22]. Occasionally, the procedure recommended by Avital and Elson [23] was followed. The tRNA from baker's yeast was prepared according to Holley et al. [24].

Crude synthetase was prepared as follows: ground seeds (100 g) were extracted with 300 ml of buffer A (0.01 M potassium phosphate buffer, pH 7.5, containing 1 mM $MgCl_2$, 10 mM β-mercaptoethanol and 10% (v/v/glycerol) at 4°C for 30 min.

The mixture was centrifuged at 20 000 *g* for 20 min and the supernatant treated with solid ammonium sulphate. Protein precipitated between 0.3 and 0.7 M ammonium sulphate saturation was

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collected by centrifugation, redissolved, dialysed against buffer A and applied on a DEAE-cellulose column (2.5 X 20 cm) equilibrated with the same buffer. The column was washed with a linear gradient (total 600 ml) of 0–0.3 M KCl in buffer A. The fractions with the highest aminoacyl-tRNA synthetase activities were pooled and used as the crude enzyme.

2.2. *Matrix*

Cellulose Whatman CF-11 powder washed with 0.5 M Na_2CO_3 and adjusted to pH 11 was activated for 30 min at 20°C with cyanogen bromide as described by Cuatrecasas [25]. CNBr (0.5–1.0 g per gram of cellulose) freshly prepared as described by Hartman and Dreder [26] was used. After activation the cellulose (4 g) was washed with ice-cold water (500 ml), 0.1 M sodium carbonate buffer (pH 10) and suspended in 20 ml of the latter. An excess of tetramethylenediamine (2 g) dissolved in water (20 ml) and adjusted with HCl to pH 10 (in some experiments tetramethylenediamine was replaced with arginine or lysine), was added to the cellulose suspension, slowly stirred for 24 hr at 4°C, and then washed with water until neutral pH of the washings was reached. The washed material was suspended in 0.1 M potassium phosphate buffer (pH 7.5, 20 ml) and mixed with dioxane solution (10 ml) of bromoacetic ester of *N*-hydroxysuccinimide prepared according to Cuatrecasas [25]. 1.2 mmole of the ester was used per 4 g of cellulose. After stirring for 30 min at 4°C, the product was collected on a Büchner funnel, washed with cold 1 M NaCl (500 ml) and water (1.5 l) and immediately used for coupling with AA-tRNA.

2.3. *Aminoacylation, binding of AA-tRNA to the matrix and releasing of tRNA*

50–150 mg of tRNA (see table 1), 1 mM Tris–HCl, pH 7.5, 0.3 mmole $MgCl_2$, 0.1 mmole ATP, 0.1 mmole amino acid, 1 mmole β-mercaptoethanol and 3–6 mg of enzyme were made up to 10 ml and incubated at 37°C for 30 min. In some experiments the incubation mixture contained ^{14}C-labelled amino acid and the time of incubation was prolonged up to 180 min. The reaction was stopped by shaking the incubation mixture (10 ml) successively with 5 portions (10 ml each) of phenol saturated with 0.1 M Tris–HCl buffer (pH 7.5) followed by 3 portions (10 ml each) of chloroform: isoamyl alcohol mixture (24:1, v/v). The aqueous phase was passed through a small column of DEAE-cellulose (10 ml). The column was washed with 0.3 M NaCl and tRNA was eluted with 1 M NaCl and precipitated with 2.5 vol of cold ethanol. The precipitate was collected by centrifugation and dissolved in 0.1 M potassium phosphate (pH 7.5) buffer (10 ml), then mixed with the freshly* prepared matrix and gently stirred for 48 hr at 4°C. The tRNA-containing matrix (4 g suspended in 10 ml of the buffer) was collected on a sintered glass funnel, washed successively with water and 1 M NaCl until the washings contained no RNA. The

* The bromoacetyl derivative is not stable; the tetramethylenediamine can be stored.

Table 1
Summary of the isolation of some specific tRNAs by covalent-binding of AA-tRNA to modified cellulose

tRNA source	Initial quantity of		Time of preparative aminoacylation (min.)	tRNA recovered after hydrolysis step (A_{260} units)	AA-acceptor activity (pmoles/ A_{260} unit)***	Yield of specific tRNA** %
	crude tRNA (A_{260} units)	specific tRNA* (A_{260} units)				
Ile, barley	2000	28	30	20	1484	53
Ile, lupin	2600	22	180	13	1526	45
Val, barley	2000	15	30	18	1271	76

* Calculated from the estimated AA-acceptor activity of crude tRNA on assumption that I A_{260} = 2 nmoles of tRNA.

** Calculated on assumption that I A_{260} unit of pure tRNA accepts 2 nmoles of amino acid.

*** The obtained specific tRNAs were assayed for acceptance of 17 other amino acids (Amersham collection CFD.103). On average they accepted much below 15 pmoles/A_{260} unit and only valine esterified the $tRNA^{Ile}$ from barley up to 75 pmoles/ A_{260}.

matrix was then suspended in 0.1 M sodium carbonate (pH 9) buffer (20 ml) and incubated for 30 min at 37°C. The mixture was neutralized with 1 M sodium acetate buffer, pH 4.7, and the released tRNA washed off with 1 M NaCl. The washings were diluted 5-fold with water and passed through a 2 ml DEAE-cellulose column. The column was washed with 0.3 M NaCl and the specific tRNA eluted with 1 M NaCl.

2.4. *Measurement of amino acid acceptor activity*

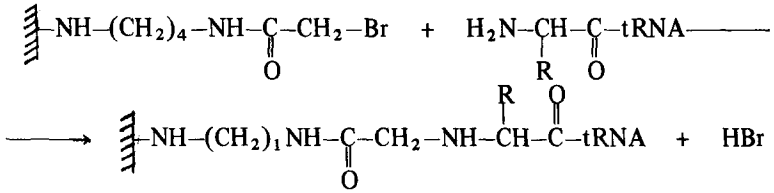
The amino acid accepting activity of tRNA was assayed by the filter paper disc method essentially as described by Yang and Novelli [27]. The incubation mixture (100 μl) contained: 2 A_{260} units of crude or 0.05 A_{260} unit of purified tRNA, 3 nmoles of ^{14}C-labelled amino acid, 10 μmoles of Tris–HCl buffer, 1 μmole of ATP, 3 μmoles of $MgCl_2$, 10 μmoles of β-mercaptoethanol and 200 μg of crude enzyme protein.

For measuring Ile and Val incorporation with the barley enzyme, pH was adjusted to 7.5, for the lupin enzyme to 8.4. Aminoacylation was performed in homologous systems, except for yeast tRNA where barley enzyme was used.

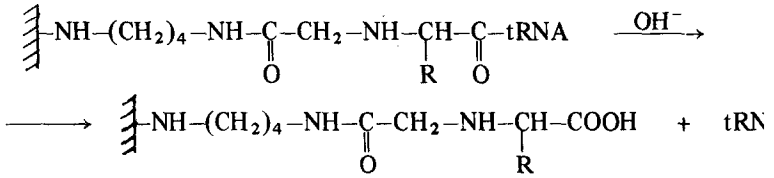
Similar enrichment of specific tRNAs was obtained from crude baker's yeast tRNA. Electrophoresis of the purified tRNAs in 7.5% polyacrylamide gel [28] showed that during the coupling and releasing steps no measurable degradation of tRNA occurred.

The high degree of tRNA purification obtained by the method suggested here is due to both the specificity of aminoacylation and the selective binding of AA-tRNA by the modified cellulose. No measurable binding was observed in a control where deaminoacylated tRNA was used instead on the charged one. Presumably in the binding of AA-tRNA to the matrix, the amino group of the amino acid residue is involved, resulting in the formation of a stable amino-bond. The experiments with ^{14}C-labelled AA-tRNA showed that after a hydrolytic splitting of tRNA from the matrix, the amino acid was still bound to the support. This as well as the lability of the ester linkage and the high activity of the released tRNA suggest that this method is based on the following reactions:

A. binding step:



B. hydrolytic step:



3. Results and discussion

The method described above enabled the isolation and substantial purification of $tRNA^{Ile}$ from barley and lupin seeds and $tRNA^{Val}$ from barley. Table 1 shows the results of a typical experiment and the amino acid acceptor activities of the isolated tRNAs.

The modified cellulose bound 70–90% of radioactivity introduced into AA-tRNA. This value, as compared with the yield of specific tRNAs, indicates that only a limited hydrolysis of AA-tRNA took place during the coupling procedure. The Ile- and Val-tRNAs, however, are among the most stable AA-tRNAs [29]. Hydrolysis of the less stable AA-tRNAs may lower the final yield of a specific tRNA

during the binding stage, thus limiting the general applicability of the method to tRNAs, whose aminoacyl esters can survive the coupling step. The same experiment also indicated that the main factor influencing the recovery of the specific tRNAs was efficiency of the preparative aminoacylation of tRNA.

The uncharged tRNA washed off the matrix after the AA-tRNA was bound retained its acceptor activity towards other amino acids. This allows for successive isolation of several different tRNAs from the same crude tRNA sample.

The removing of the noncovalently bound tRNA from the matrix could not be achieved by washing with water alone and 1 M NaCl had to be used. This indicates a strong ionic interaction of tRNA and the carrier. Similarly strong ionic interaction was observed when CNBr-activated Sepharose was used instead of cellulose. The anion-exchange properties of CNBr-activated Sepharose was also observed by other authors [30] and are probably due to multidirectional reactions of polysaccharides with CNBr [31].

Important for the efficient binding of AA-tRNA to the modified cellulose was the 'extension arm' introduced between the matrix and the active bromoacetyl group. The efforts taken to bind AA-tRNA directly to CNBr-activated cellulose were unsuccessful.

If governed by the same steric factors as observed by Cuatrecasas [25], for affinity chromatography, this might indicate that in AA-tRNA the amino acid residue does not protrude from the tRNA molecule.

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